

Full Review

Genetic diseases of renal phosphate handling

Carsten A. Wagner, Isabel Rubio-Aliaga, Jürg Biber and Nati Hernando

Institute of Physiology and Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

Correspondence and offprint requests to: Carsten A. Wagner; E-mail: Wagnerca@access.uzh.ch

ABSTRACT

Renal control of systemic phosphate homeostasis is critical as evident from inborn and acquired diseases causing renal phosphate wasting. At least three transport proteins are responsible for renal phosphate reabsorption: NAPI-IIa (SLC34A1), NAPI-IIc (SLC34A3) and PIT-2 (SLC20A2). These transporters are highly regulated by various cellular mechanisms and factors including acid–base status, electrolyte balance and hormones such as dopamine, glucocorticoids, growth factors, vitamin D₃, parathyroid hormone and fibroblast growth factor 23 (FGF23). Whether renal phosphate wasting is caused by inactivating mutations in the *NAPI-IIa* transporter is controversial. Mutations in the *NAPI-IIc* transporter cause hereditary hypophosphatemic rickets with hypercalciuria. Besides the primary inherited defects, there are also inherited defects in major regulators of phosphate homeostasis that lead to alterations in phosphate handling. Autosomal dominant hypophosphatemic rickets is due to *FGF23* mutations leading to resistance against its own degradation. Similarly, inactivating mutations in the *PHEX* gene, which causes FGF23 inactivation, cause X-linked hypophosphatemia due to renal phosphate losses. In contrast, mutations in galactosamine:polypeptide *N*-acetyl-galactosaminyltransferase, responsible for O-glycosylation of FGF23, or in *klotho*, a cofactor for *FGF23* signalling result in hyperphosphatemia. Acquired syndromes of renal phosphate wasting, hypophosphatemia and osteomalacia (tumour-associated osteomalacia) can be due to the excessive synthesis or release of phosphaturic factors (FGF23, FGF-7, MEPE and sFRP4) from mesenchymal tumours.

Keywords: bone, FGF23, kidney, phosphate, PTH

INTRODUCTION

About 85% of total phosphate is deposited in bone and ~10% in soft tissues, the remaining 2–3% is found in serum,

constituting a freely exchangeable and tightly regulated phosphate pool. Serum phosphate levels in adults are maintained in a narrow range of ~0.8–1.45 mmol/L (2.4–4.5 mg/dL). Deviations as found in patients with hypophosphatemia or hyperphosphatemia can cause severe disturbances of cellular and organ function such as ATP depletion, rickets, osteomalacia, anaemia or excessive tissue calcifications, nephrolithiasis, arteriosclerosis and increased risk of cardiovascular morbidity and mortality [1–3].

Phosphate homeostasis is the product of intestinal uptake of phosphate (in the range of 0.9–1 g/day) and reabsorption of phosphate from urine (in the range of 60–80%). Both intestinal uptake and renal reabsorption are mediated by sodium-dependent phosphate cotransporters (Na⁺/P_i-cotransporters) belonging to the *SLC20* and *SLC34* gene families of solute carriers [4, 5]. Their activity and expression is tightly regulated by a large variety of hormones and metabolic factors adjusting uptake and excretion to short and long-term body requirements. Inborn errors are caused by mutations in phosphate transporters and in several genes that encode factors directly and indirectly involved in their regulation. The identification of these genes and elucidation of their functions have greatly contributed to our present understanding of how phosphate homeostasis is achieved. This review will give a brief overview over physiological function and regulation of renal phosphate transporters. For more extensive reviews, the reader is referred to a number of recent reviews and book chapters [5–9]. The second part of this review will discuss mutations in several genes that directly or indirectly participate in (renal) control of phosphate balance.

General aspects of renal phosphate handling

About 80% of filtered phosphate is reabsorbed from urine under normal dietary phosphate intake by the earlier convoluted parts of the proximal tubule of mostly juxtamedullary nephrons [4]. Phosphate absorption is mediated by sodium-dependent

cotransporters for inorganic phosphate (Pi) located in the brush border membrane.

Three members of the Type II Na⁺-dependent phosphate cotransporter family have been identified and classified in the *SLC34* solute carrier gene family [4]. *SLC34A1* (*NaPi-IIa*) is predominantly expressed in kidney mediating ~70–80% of overall phosphate reabsorption as suggested by a *Slc34a1* deficient mouse model [10]. Another member of this family, *SLC34A3* (*NaPi-IIc*), is also almost exclusively found in kidney [11]. *SLC34A2* (*NaPi-IIb*) is mostly expressed in small intestine and in a number of other organs including testis, lung, liver and lactating mammary glands [12].

Also, sodium-dependent phosphate transporters from the *SLC20* family are expressed in the kidney. *PIT-2* (*SLC20A2*) is localized in the brush border membrane of the proximal tubule whereas the exact site of expression of *PIT-1* in kidney is unclear [13]. *PIT-2* is widely expressed in most tissues. Its exact role in renal phosphate reabsorption has not been clarified but simultaneous genetic deletion of *NaPi-IIa* and *NaPi-IIc* in mice reduced renal phosphate reabsorption by >90%, suggesting that its contribution to overall phosphate reabsorption may be minor [14]. *PIT-2* is regulated by dietary phosphate intake, acid–base status and parathyroid hormone (PTH) [15, 16]. Mutations in *PIT-2* have been described in patients with idiopathic basal ganglia calcification [17].

SLC34A1 (NaPi-IIa) and SLC34A3 (NaPi-IIc)

The human *SLC34A1* gene lies on chromosome 5q35 [18], is ~13 kb in length and consists of 13 exons and 12 introns [19]. Recently, a model for the protein structure of *NaPi-IIa* was suggested based on homology modelling and detailed structure–function studies [20].

The human *SLC34A3* gene spans ~5 kb containing 13 exons and lies on chromosome 9q34 [21, 22]. Human *NaPi-IIc* contains 599 amino acids [21, 22]. *NaPi-IIc* expression in mouse is stimulated in response to low-dietary phosphate intake [23]. Also, dietary Pi-responsive elements (potential TFE-3-binding sites) are located at –1846 and –1822 [23].

The transport functions of *SLC34A* transporters and their structure–function relationship have been extensively studied elucidating important features such as cotransport mode, electrogenicity or voltage and pH-dependency [4]. Collectively, these data demonstrate that *NaPi-IIa* transports three sodium ions together with one divalent phosphate ion (HPO_4^{2-}) per transport cycle. This transport mode is electrogenic [24, 25]. The transporter homodimerizes but the functional unit is a monomer [26, 27]. The Type IIc cotransporter transports only two sodium ions per phosphate and is electroneutral, indicating that divalent phosphate is its preferred substrate [28].

Regulation of renal phosphate reabsorption

A variety of factors affects renal phosphate handling as listed in Table 1 [4, 29]. Alterations in the number of phosphate transporter molecules in the brush border membrane represent the main mechanism leading to changes in urinary phosphate excretion [29]. The cellular and molecular mechanisms, by which *NaPi-IIa* and *NaPi-IIc* are regulated have been

Table 1. Factors affecting phosphate reabsorption in the proximal tubule

| Factors that decrease Pi reabsorption | Factors that increase Pi reabsorption |
|---------------------------------------|---------------------------------------|
| Acidosis | Alkalosis |
| High-dietary Pi intake | Low-dietary Pi intake |
| Parathyroid hormone/cAMP | Parathyroidectomy |
| Atrial natriuretic peptide/cGMP | Thyroid hormone |
| Glucocorticoids | 1 α ,25(OH) $_2$ D $_3$ |
| Dopamine | Growth hormone(s) |
| Fibroblast growth factor 23 | Insulin-like growth factor |
| MEPE | |
| Frizzled-related protein 4 | |

For explanation and references see text.

extensively studied for PTH and changes in dietary phosphate intake (for review, [4, 5]).

PTH rapidly induces phosphaturia within minutes by decreasing apical phosphate transport activity. Activation of PLC by apical PTH receptors leads to protein kinase C dependent stimulation of ERK1/2 kinases and internalization of *NaPi-IIa* [29–31]. *NaPi-IIa*, apical PTH receptors and PLC β 1 are organized in a macromolecular complex via the PDZ-scaffold protein NHE regulating factor 1 (NHERF1) [32–34]. In contrast, basolateral PTH receptors are linked to adenylate cyclase, protein kinase A (PKA) and ERK1/2 [31]. *NaPi-IIa* is not phosphorylated but dissociates from NHERF1, which is phosphorylated in response to PTH [35, 36]. A similar mechanism has been proposed for fibroblast growth factor 23 (FGF23)- and dopamine-induced endocytosis of *NaPi-IIa* [4].

The regulation of renal phosphate reabsorption by FGF23 is mediated by several mechanisms: FGF23 reduces circulating levels of active 1,25(OH) $_2$ vitamin D $_3$ by reducing expression of the activating enzyme CYP27B1 and by increasing expression of the inactivating enzyme CYP24A1 [37]. Lower active vitamin D $_3$ reduces the stimulation of renal and intestinal phosphate (re)absorption. Moreover, renal phosphate reabsorption is directly reduced by FGF23 through downregulation of *NaPiIIa* and *NaPiIIc*. Two alternative but not exclusive pathways have been proposed. FGF23 binds to FGF1c receptors in the distal convoluted tubule, the main site of *klotho* expression in kidney leads to ERK1/2 phosphorylation and the distal convoluted tubule then signals to the proximal tubule to internalize *NaPi-IIa* and *NaPi-IIc* [38]. Alternatively, FGF23 may bind to FGF23 receptors in the proximal tubule and lead to phosphorylation of NHERF1 via Sgk1 [39]. Also, a direct regulation of *NaPiIIa* activity by *klotho* without FGF23 has been demonstrated where *klotho* causes cleavage and inactivation of *NaPi-IIa* in the brush border membrane and subsequent internalization [40].

An acute reduction in *NaPi-IIa* transporters is mediated by the internalization from the apical brush border membrane via the same route as receptor-mediated endocytosis, requiring megalin and involving clathrin-coated vesicles and early endosomes [41, 42]. Consequently, *NaPi-IIa* is routed to lysosomes for degradation [43, 44]. The regulation of *NaPi-IIc* has been studied in less detail: in response or high phosphate *NaPi-IIc* downregulation occurs but is slower than for *NaPi-IIa* [16, 45].

Renal phosphate reabsorption closely matches dietary phosphate intake being high during dietary phosphate restriction and decreasing with high phosphate intake [11, 46–48]. In animals lacking PTH and/or 1,25(OH)₂-vitamin D₃, the adaptive response of NaPi-IIa and NaPi-IIc is partially preserved, indicating that other factors may be responsible for sensing and mediating changes in dietary phosphate intake [49–51].

PRIMARY INHERITED DEFECTS IN RENAL PHOSPHATE HANDLING

SLC34A1 (NAPI-IIa): hypophosphatemic nephrolithiasis/osteoporosis-1 and Fanconi renotubular syndrome-2

Two reports addressed patients with mutations in the *SLC34A1/NAPI-IIa* gene. In the first report, two patients with urolithiasis or bone demineralization and persistent idiopathic hypophosphatemia with lower maximal renal phosphate reabsorption (TmP/GFR) were presented [52]. Single nucleotide changes resulting in missense mutations (A48F and V147M) were detected. These mutations were found only on one allele per patient suggesting a dominant effect [52]. Expression of the mutant *NAPI-IIa* transporters in oocytes has demonstrated reduced phosphate transport activity, a dominant negative effect on wild-type *NAPI-IIa* cotransporters, and reduced affinity for phosphate [52]. However, these data were controversially discussed. *SLC34A1* mutations were not confirmed in other kindreds with similar symptoms, relatives of patients had not been genetically investigated and the expression assay showed only low expression even for the wild-type transporter rendering a kinetic analysis very difficult. Moreover, expression of the reported mutant *NAPI-IIa* cotransporters in *Xenopus* oocytes and the renal OK cell line did not show any evidence for altered subcellular localization, levels of expression or reduced substrate affinity [53], but showed a lower transport activity. Yet, this decrease cannot fully explain the massive phosphaturia observed. Therefore, it was suggested that the nucleotide changes may represent only polymorphisms. A similar conclusion was derived from screening a large number of pedigrees of calcium stone formers with low TmP/GFR [54]. Even though a number of mutations including an N-terminal deletion of seven amino acids (91del7) in the *NAPI-IIa* gene were found, no correlation with the clinical phenotype could be established. Two of the mutations had lower phosphate transport activity after expression in *Xenopus* oocytes, most likely due to decreased membrane abundance. However, the reduction in transport rates was mild and importantly, only one allele was found to be mutated in stone forming patients. The interpretation that these mutations may represent rather relatively common polymorphisms was further supported by the fact that these gene alterations were also found in several subjects with normal renal phosphate excretion. Thus, polymorphisms in the *NAPI-IIa* gene may be relatively common and their relevance for renal phosphate handling may require further clarification (Table 2).

A second report described siblings from a consanguineous Arab Israeli family with hypophosphatemic rickets, multiple fractures and stunted growth [55]. The presence of a Fanconi-like syndrome was suggested, but hallmarks of a generalized proximal tubule dysfunction are not evident from data reported. The patients suffered from hypercalciuria due to elevated serum 1,25(OH)₂D₃ levels. Hypophosphatemia was refractory to vitamin D therapy. Genetic analysis detected a homozygous in-frame duplication of 21 bp leading to insertion of seven additional amino acids at position G154-V160. Heterozygous carriers of the mutation were clinically normal suggesting that the mutation did not exert a dominant negative effect. Expression of the mutant protein in *Xenopus* oocytes abolished all phosphate-induced currents whereas expression in renal OK cells indicated failure of the mutant protein to reach the plasma membrane.

Thus, homozygous mutations in *SLC34A1* may be responsible for renal phosphate wasting but whether this is only the case for this specific mutation and may be due to toxic effects of the mutant protein for proximal tubule functions or whether mutations in this phosphate transporter can provide evidence for the biological and clinical importance in human kidney function remains to be established (Table 3).

SLC34A3 (NAPI-IIc): hereditary hypophosphatemic rickets with hypercalciuria

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) (OMIM #241530) is caused by mutations in the *SLC34A3* gene encoding for *NAPI-IIc* [21, 22]. The disease was first described in a Bedouin family [56] and is characterized by renal phosphate wasting causing hypophosphatemia and secondary rickets, with elevated serum alkaline phosphatase. Serum levels of 1,25(OH)₂-vitamin D₃ are high, PTH and FGF23 are low, resulting in enhanced intestinal calcium absorption and subsequent renal excretion (hypercalciuria) promoting nephrolithiasis or nephrocalcinosis in some patients [21, 22, 56, 57]. Long-term phosphate supplementation appears to reverse the clinical and biochemical abnormalities in HHRH.

The *SLC34A3* gene is localized on chromosome 9q34. Observed mutations in HHRH include frame shifts in the open reading frame with subsequent translation of sequences unrelated to NaPiIIc and premature termination, missense mutations, intronic deletions and nucleotide changes in a putative splice site [21, 22]. Inheritance follows an autosomal recessive pattern [21, 22]. Some compound heterozygous patients carrying two different missense mutations were also identified [21]. However, carriers with only one allele mutated show normal phosphate balance and excretion but borderline or elevated renal calcium excretion [21, 22].

Since *NAPI-IIc* mutations are associated with a severe renal phosphate leak, *NAPI-IIc* may have a more important role in human kidney function than in rodents. *NAPI-IIc* function in man remains important during adulthood in contrast to rodents where NaPi-IIc may play an important role only during growth [11]. Accordingly, two mouse models of *Slc34a3* deficiency show only mild hypercalciuria without hyperphosphaturia or even no symptoms in the case of a kidney-specific gene deletion [58, 59].

Table 2. Genes/diseases causing altered renal phosphate handling

| Gene | Chromosome/ OMIM | Disease name | Major symptoms | Inheritance | Tissue distribution of protein |
|----------------------|---------------------------------------|--|--|--|---|
| SLC34A1 (NaPiIIa) | 5q35 OMIM#612286 OMIM#613388 | Hypophosphatemic nephrolithiasis/osteoporosis-1 (NPHLOP1) Fanconi renotubular syndrome-2 (FRTS2) | Hypophosphatemia, urolithiasis, osteoporosis? Fanconi-like syndrome | Autosomal dominant Autosomal recessive | Kidney (proximal tubule) |
| SLC34A3 (NaPiIIc) | 9q34 OMIM#241530 | HHRH | Hypophosphatemia, hyperphosphaturia, rickets, Hypercalciuria | Autosomal recessive | Kidney (proximal tubule) |
| SLC9A3R1 (NHERF1) | 17q25.1 OMIM#612287 | Hypophosphatemic nephrolithiasis/osteoporosis-2 (NPHLOP2) | Normal low FGF23, high 1,25-D ₃ Hypophosphatemia Hyperphosphaturia Normal-low PTH Mildly-elevated 1,25-D ₃ Nephrolithiasis | Autosomal dominant | Kidney, intestine |
| FGF23 | 12p13.3 OMIM#193100 OMIM#211900 | ADHR FTC | Hyperphosphaturia Hypophosphatemia Rickets/osteomalacia Normal 1,25-D ₃ High FGF23 | Autosomal dominant | Mainly bone |
| Klotho | 13q13.1 OMIM#211900 | FTC Hypophosphatemic rickets with hyperparathyroidism | Hyperphosphatemia hypercalcemia Heterotopic calcifications high-intact FGF23 high PTH Hypophosphatemia Hyperphosphaturia High FGF23 High PTH | Autosomal recessive Translocation, dominant | Kidney, parathyroid gland, brain |
| PHEX | Xp22.2-p22.1 OMIM#307800 | XLH | Hyperphosphaturia Hypophosphatemia Rickets/osteomalacia Normal vitamin D High FGF23 | X-linked | Mainly bone |
| GALNT3 | 2q24-q31 OMIM#211900 | FTC | Hyperphosphatemia heterotopic calcification | Autosomal recessive | Pancreas, testis |
| GNAS1 | 20q13.2 OMIM#174800 | FD (McCune-Albright syndrome) | Hyperphosphaturia, high-FGF23 levels, polyostotic FD, pigmentation, thyrotoxicosis, Cushing | Autosomal dominant, mosaicism | Ubiquitous |
| FAM20c | 7p22.3 OMIM#259775 | Raine syndrome Osteosclerotic bone dysplasia | Increased bone density, periosteal bone growth Skull deformation | Autosomal recessive | Bone, kidney, mammary gland, and others |
| FGFR1 | 8p11.2-p11.1 OMIM#166250 | Osteoglophonic dysplasia | Hypophosphatemia Hyperphosphaturia Craniosynostosis | Autosomal dominant? | Ubiquitous, bone, kidney |
| DMP1 | 4q21 OMIM#241520 | Autosomal recessive hypophosphatemic rickets Autosomal recessive hypophosphatemia | Hypophosphatemia Hyperphosphaturia Rickets, osteomalacia | Autosomal recessive | Ubiquitous, Bone, heart, kidney |

For explanation and references see text.

Table 3. Acquired forms of renal phosphate wasting

| Gene/factor | Chromosome/OMIM | Disease | Symptoms | Occurrence | Tissue distribution |
|-------------|---------------------------|---------|---|--------------|---------------------|
| sFRP4 | 7p14-p13 OMIM606570 | TIO | Hypophosphatemia Hyperphosphaturia Osteomalacia | Spontaneous? | Tumour bone |
| FGF7 | 15q15-q21.1 OMIM148180 | TIO | Hypophosphatemia Hyperphosphaturia Osteomalacia | Spontaneous? | Tumour |
| MEPE | 4q21.1 OMIM605912 | TIO | Hypophosphatemia Hyperphosphaturia Osteomalacia | Spontaneous? | Tumour, bone |

SLC9A3R1 (NHERF1): Na⁺/H⁺ exchanger regulating factor 1: hypophosphatemic nephrolithiasis/osteoporosis-2

NHERF1 mutations were detected in eight patients with mildly reduced TmP/GFR, slightly lower serum phosphate levels, nephrolithiasis in four of eight patients, lower bone mineral density in one patient, normal to low PTH and calcium values and 1,25(OH)₂D₃ just above the normal range in all patients. Four NHERF1 mutations (E68A, L110V, R153Q and E225K) were found. All patients were heterozygous [60, 61]. The mechanisms by which mutant NHERF1 may cause phosphaturia may be site specific. In the case of the L110V, R153Q and E225K mutations, PTH- and cAMP-dependent inhibition of phosphate transport may be increased, whereas the E68A mutation may reduce the stability of the NaPiIIa transporter in the plasma membrane leading, in all cases, to lower transport activities [60, 61].

DEFECTS IN RENAL PHOSPHATE HANDLING SECONDARY TO EXTRARENAL INHERITED DEFECTS

Fibroblast growth factor 23

FGF23 is primarily expressed in osteocytes [62–64]. Synthesis and release of FGF23 from bone are increased during hyperphosphatemia or high intake of phosphate [9, 65]. The major functions of FGF23 are the inhibition of renal phosphate reabsorption by downregulation of *NaPi-IIa* and *NaPi-IIc* expression and activity and by reducing the synthesis of active vitamin D₃ through lowering expression of the renal *CYP27B1* (1,25-alpha hydroxylase) and increasing *CYP24A1* (1,24,25 hydroxylase). Lower concentrations of vitamin D₃ decrease intestinal phosphate absorption. In addition, FGF23 suppresses PTH secretion. The effects of FGF23 on phosphate homeostasis are mediated by fibroblast growth factor receptor 1c (FGFR1c) and FGFR4 receptors and require klotho as an obligatory co-ligand converting the low-affinity receptors into high-affinity FGF23 receptors [9, 66].

Synthesis of FGF23 in osteocytes is regulated by a number of factors including PTH, 1,25 vitamin D₃ and a cascade of proteins consisting of PHEX, 7B2/PC2, BMP1 and Dentin matrix protein 1 (DMP1). PTH and 1,25 vitamin D₃ transcriptionally stimulate FGF23 production by mechanisms involving PKA/Wnt and the VDR receptor, respectively [67, 68]. Activation of this cascade increases proteolytic processing of DMP1 into N- and C-terminal fragments, from which the latter is proposed to blunt the transcription of FGF23 (for review, see [69]).

FGF23 is degraded by C-terminal cleavage most likely by subtilisin-like proprotein convertases [70]. FGF23 possesses a recognition site for cleavage by subtilisin-like proprotein convertases consisting of an RXXR motif at position 176. A recent report suggested that the PC2 proprotein convertase, complexed with its chaperon 7B2, may mediate this process [71].

FGF23-activating mutations: autosomal dominant hypophosphatemic rickets

Autosomal dominant hypophosphatemic rickets (ADHR) (OMIM # 193100) is characterized by rickets, hypophosphatemia,

hyperphosphaturia, fatigue, bone pain and lower bone deformities in face of inappropriately low or normal vitamin D₃ levels. ADHR is caused by mutations destroying the RXXR cleavage motif mentioned above, causing excessive levels of active FGF23 [72]. Similarly, injection of mice with wild-type *Fgf23* or *Fgf23*^{R179Q} caused ADHR-like symptoms in mice. Mutant *Fgf23* caused hypophosphatemia, hyperphosphaturia, reduced intestinal phosphate absorption and suppressed serum vitamin D₃ levels. In serum, high levels of mutant *Fgf23* were detected, whereas wild-type *Fgf23* was degraded [73]. Transgenic mice for *Fgf23*^{R176Q} display typical ADHR symptoms and show signs of secondary hyperparathyroidism [74]. Thus, ADHR is caused by mutations in a motif important for cleavage and degradation of FGF23 resulting in excessive FGF23 signalling.

Loss-of-function FGF23 mutations: familial tumoral calcinosis

Familial tumoral calcinosis (FTC) (OMIM # 211900) is the mirror image of phosphate wasting diseases such as ADHR, tumour-induced osteomalacia (TIO) and X-linked hypophosphatemia (XLH). FTC is characterized by hyperphosphatemia, reduced renal phosphate excretion, ectopic calcifications and inappropriately normal or elevated 1,25(OH)₂ vitamin D₃ levels. In a subset of FTC patients, a homozygous 211A-G transition in the *FGF23* gene was identified, resulting in the substitution at an evolutionarily conserved serine to glycine (S71G) [75]. This mutation may cause loss of function [75]. Patients have abnormally low-FGF23 plasma concentrations. Mutant FGF23 protein expressed *in vitro* was not secreted and retained intracellularly [75].

Fibrous dysplasia (McCune–Albright syndrome)

Fibrous dysplasia (FD) (OMIM #174800) is caused by non-inherited genetic somatic activating missense mutations in the α-subunit of the stimulatory G protein, G_s [76, 77]. FD is characterized by abnormalities in bone (monostotic or polyostotic FD), in skin (pigmentation) and in the endocrine system (thyrotoxicosis, pituitary gigantism and Cushing syndrome). The severity of disease and particularly the association with skin and endocrine symptoms shows a wide variability. Renal phosphate wasting is detected in ~50% of patients [78]. FGF23 levels are elevated and caused by a large mass of FGF23-producing cells in fibrous bone lesions. Abnormal high-FGF23 levels are caused by an increase in FGF23-producing cells but not by abnormal production of FGF23 *per se* [62, 79].

Klotho: FTC and hypophosphatemic rickets with hyperparathyroidism

Mutations in the alpha-klotho (*KL*) gene can be another reason for FTC besides mutations in galactosamine:polypeptide *N*-acetyl-galactosaminyltransferase (*GALNT3*) or loss-of-function mutations in *FGF23*. A single patient with a missense mutation was identified [80]. The mutation is predicted to affect the glucosidase domain of klotho, which is involved in the regulation of TRPV5 calcium channels [81]. Moreover, reduced expression and glycosylation of klotho was demonstrated, impairing the ability to interact with FGF23 and the

FGF1R receptor. Accordingly, the patient had hyperphosphatemia, increased vitamin D₃ and PTH levels and ectopic calcifications of vessels.

The mirror image was described in another single case of a balanced translocation between chromosomes 13 and 9 at a position on chromosome 13 adjacent to the alpha-klotho gene. The translocation massively increased circulating klotho levels leading to hypophosphatemia, hyperphosphaturia, hypercalcaemia, elevated PTH and FGF23 and low vitamin D₃ [82]. The elevated FGF23 levels may be explained by a feedback loop, by which circulating alpha klotho stimulates bone FGF23 expression [83].

DMP1: autosomal recessive hypophosphatemic rickets

Patients with mutations in *DMP1* suffer from hypophosphatemia due to hyperphosphaturia with inappropriately normal levels of 1,25(OH)₂ vitamin D₃ [84, 85]. *DMP1* belongs to the large SIBLING family (small integrin-binding ligand, N-linked glycoproteins) of extracellular matrix proteins and is mainly co-expressed with FGF23 in bone. The similar phenotype of patients with XLH and autosomal recessive hypophosphatemia with rickets suggested a link to FGF23. Indeed, in patients with loss of intact *DMP1*, FGF23 levels in serum were normal or elevated [84, 85]. *DMP1* (in particular the C-terminal fragment) blocks the transcription of FGF23; therefore, its absence may result in the release of this negative control leading to higher FGF23 production. The hypophosphatemia in *DMP1* deficient patients causes severe rickets in children or osteomalacia in adults [84, 85], which is characterized by abnormal amounts of osteoid indicating defective mineralization and maturation of bone [85]. Similarly, a mouse model deficient for *DMP1* shows hypophosphatemia, hyperphosphaturia, rickets, retarded skeletal growth with abnormal mineralization, disturbed lacunocanicular organization and defective osteoblast to osteocyte differentiation [85].

Tumour-induced osteomalacia

TIO is a rare paraneoplastic syndrome mostly associated with mesenchymal tumours releasing (a) phosphaturic factor (s). Symptoms include renal phosphate wasting causing hypophosphatemia, osteomalacia and abnormal vitamin D metabolism [86]. Surgical removal of the tumour reverses symptoms. In contrast to syndromes of hyperparathyroidism or humoral hypercalcaemia of malignancy, the plasma concentrations of calcium, PTH and PTH-related protein are in the normal range. Several proteins, such as FGF23, sFRP4, FGF-7 and matrix extracellular phospho-glycoprotein (MEPE), have been identified that are produced and secreted from tumours from patients with TIO. Some of these proteins have been shown to regulate phosphate transport *in vitro* and/or *in vivo*.

Secreted frizzled-related protein-4 (sFRP-4) is highly upregulated in tumour tissue from patients with renal phosphate wasting [87] and inhibits phosphate transport in the renal OK cell line as well as *in vivo* [87, 88]. However, mice lacking *sfrp4* do not show any abnormalities of systemic phosphate balance [89]. Thus, the relevance of sFRP4 for phosphate homeostasis remains to be further clarified.

MEPE: oncogenic hypophosphatemia

MEPE, a glycosylated protein of about 60 kDa, was initially cloned from tumour tissue obtained from a patient with oncogenic hypophosphatemia [90]. Bone cells (osteoblasts, osteocytes and odontoblasts) are the major source of MEPE. MEPE-like *DMP1* is another member of the SIBLING family of extracellular matrix proteins involved in bone regulation. Injection of MEPE into intact mice results in hypophosphatemia, hyperphosphaturia and mild increases in circulating 1,25(OH)₂ vitamin D₃ levels [91]. Moreover, implantation of MEPE-producing CHO cells into nude mice caused renal phosphate wasting, whereas *MEPE* deficient mice have higher bone density [92]. The interactions between MEPE and other hormones regulating phosphate homeostasis and handling require more investigation.

PHEX: XLH rickets

XLH rickets (OMIM #307800) is the most common heritable form of rickets with a prevalence of ~1 in 20 000. The disease is characterized by hypophosphatemia due to excessive renal phosphate wasting leading to rickets, lower extremities deformity, short stature, bone and joint pain, enthesopathy and dental abscesses. Vitamin D₃ levels are inappropriately normal or even low [93]. The disorder is inherited in a dominant manner. Positional cloning identified the *PHEX* gene (*PH*osphate regulating gene with homology to *Endopeptidases* on the X chromosome) in XLH patients [93]. Several mouse models of XLH are available: *Hyp*, *Gy* and *Ska1* mice resembling XLH and which were later shown to have mutations in the mouse *Phex* homologue [94–96]. The X-linked *Hyp* mouse model demonstrates a defect in proximal tubular phosphate absorption, decreased expression of *NaPi-IIa* and *NaPi-IIc* [97–99]. Serum FGF23 levels are highly elevated in XLH patients [100]. Crossing of hypophosphatemic *Hyp* mice with hyperphosphatemic *Fgf23* deficient mice produced hyperphosphatemic mice that showed exactly the same phenotype as *Fgf23* null mice, indicating that both mutations affect the same system and that FGF23 may act downstream of *PHEX* [101]. Observations in *Hyp* and *FGF23* null mice indicate that *PHEX* and FGF23 may regulate each other's expression levels and that loss of *PHEX* may lead to higher expression levels of FGF23 [63, 65]. Although originally proposed, *PHEX* seems not to mediate direct cleavage of FGF23 [63, 70]. Instead, *PHEX* may activate the PC2 proprotein convertase by promoting the transcription of its 7B2 chaperon [71]. Transfection of osteoblast with 7B2.PC2 promoted cleavage of FGF23, whereas depletion of 7B2 mRNA reduced FGF23 cleavage and increased its transcription. The activated FGF23 transcription seems to result from an impaired proteolytic processing of *DMP1* (see above). In agreement with this model, the mRNA levels of 7B2 are reduced in bones from *Hyp* mice [71].

UDP-N-acetyl- α -D GALNT3: FTC

Homozygous loss-of-function mutations of the UDP-N-acetyl- α -D *GALNT3*, a glycosyltransferase involved in mucin-type O-glycosylation, underlie also FTC (OMIM # 211900) [102, 103]. However, patients carrying only one mutated allele

appear to also have mild symptoms, leading to the initial description of FTC as an autosomal dominant disease [104]. Because inactivating mutations in *FGF23* also cause FTC and *FGF23* has some O-glycosylation sites within the subtilisin-recognition sites, it had been speculated that *GALNT3* is critical for *FGF23* glycosylation and full function. Accordingly, *in vitro* secretion of *FGF23* from CHO cells deficient in O-glycosylation is impaired, and cotransfection of *GALNT3* markedly increases O-glycosylation and secretion of *FGF23*. Thus, *GALNT3* may play an important role in *FGF23* secretion by mediating its O-glycosylation [105]. This function may also explain how loss of function mutations in either *FGF23* or *GALNT3* can produce the same disease, FTC.

Fibroblast growth factor receptor 1: osteoglophonic dysplasia

Osteoglophonic dysplasia (OMIM #166250) is caused by mutations in the *FGFR1* that result in a gain-of-function and activation of the receptor [106]. Patients suffer from craniosynostosis, prominent supraorbital ridge and depressed nasal bridge, as well as from rhizomelic dwarfism and nonossifying bone lesions. Several missense mutations in the *FGFR1* gene were identified in four patients. Three patients were hypophosphatemic due to massive renal phosphate wasting [106]. One patient had inappropriately high-*FGF23* levels, two patients had high 1,25(OH)₂ vitamin D₃ levels. As two related patients carried the Y372C *FGFR1* mutation. The activity of the mutant receptor was highly increased suggesting that overactivity of the *FGFR1* receptor is responsible for this disease [106]. The *FGFR1* receptor mediates the downregulation of NAPI-IIa and NAPI-IIc by *FGF23*, whereas the effects of *FGF23* on vitamin D₃ metabolism may involve the *FGFR4* receptor [66].

FAM20C: Raine syndrome (osteosclerotic bone dysplasia)

Raine syndrome is caused by mutations in the protein kinase FAM20C, which resides in the Golgi apparatus and is secreted [107, 108]. Patients develop generalized higher bone density with characteristic changes and enhancement of the ossification of the skull, cerebral calcification and in some cases hypoplastic lungs. The disease is often lethal in the first weeks after birth but patients with longer survival reaching into puberty have been described. The renal phenotype of patients with Raine syndrome has not been reported in detail. Targets phosphorylated by FAM20C are casein, osteoprotegerin, DMP1 and MEPE, members of the SIBLING family. In Fam20c deficient mice, hypophosphatemic rickets was observed possibly due to dysregulation of *FGF23* (which is very high) due to lack of DMP1 phosphorylation [109].

CONCLUSION AND OUTLOOK

Renal and extrarenal control of systemic phosphate homeostasis requires a complicated network of regulatory factors, phosphate transporters in kidney, intestine and bone and intracellular protein–protein interactions. Rare human genetic disorders and mouse genetics have greatly contributed to our

current understanding of (renal) phosphate homeostasis. Importantly, population-based genome-wide association studies identified *SLC34A1* and *FGF23* as important determinants of plasma phosphate levels [110]. Moreover, the highly elevated *FGF23* levels in patients with various types of chronic kidney disease and the potential link between *FGF23* and cardiovascular disease in these patients underline even further the biological importance of this homeostatic system for health and disease. Nevertheless, our understanding of key elements of this system is incomplete and we require deeper insights on how organs and cells sense phosphate and how changes in systemic phosphate levels can trigger disease. The identification of key players through genetics may also pave the way for therapies targeting disturbed phosphate balance in both rare inherited disorders as well as in more common diseases such as chronic kidney disease.

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CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part.

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